Alzheimer's disease-derived *Phocaeicola dorei* contributes to cognitive decline in a strain-specific manner

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Background

The human gut microbiota undergoes substantial compositional and functional shifts with aging and Alzheimer's disease (AD). While associations between bacterial species and disease risk are increasingly recognized, functional heterogeneity at the strain level remains poorly understood. Here, we examined whether strain-level variation within *Phocaeicola dorei*, a common human gut commensal bacterium that is elevated in AD, differentially influences host immunity and neurodegenerative outcomes.

1. AD-*B. dorei* fails to stimulate Aβ uptake *in vitro*

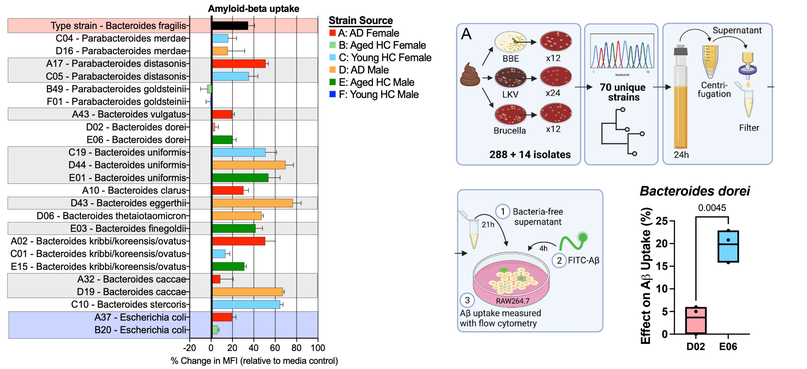


Figure 1. Bacteroides strain-specific effects on Aβ uptake. Bacteria were isolated from AD and HC subjects. Bacterial supernatants were used to stimulate a macrophage cell line for 21 hours at 1% volume, then FITC-Aβ was added for 3 hours, and clearance was assessed by flow cytometry. The change in mean fluorescent intensity (MFI) of Aβ-FITC was analyzed to determine effect on Aβ uptake. ** P<0.01

2. *B. dorei* strain-specific effects on cognition in APP/PS1 mice

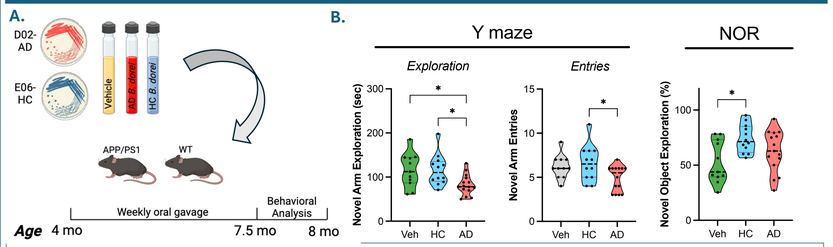


Figure 2. HC and AD-*B. dorei* have differential effects on cognition in 7.5-month-old APP/PS1 females. **A)** Female APP/PS1 mice were administered HC- or AD-*B. dorei* or vehicle control from 3.5 to 7.5 months of age before undergoing cognitive testing. **B)** Novel arm exploration and entries on the blocked Y-maze and Novel object exploration on the Novel Object Recognition test. *P<.05, one-way ANOVA with Tukey's MTC.

3. AD-*B. dorei* promotes an inflammatory peripheral response

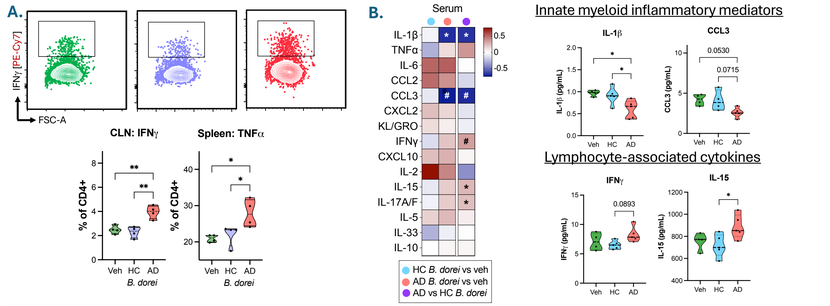


Figure 3. AD-*B. dorei* immune effects. A) Cytokine producing CD4 T cells harvested from the spleen and cervical lymph nodes, followed by stimulation with PMA ionomycin. Stimulated T cells underwent surface and intracellular staining before flow cytometric analysis. **B)** Serum samples underwent MSD multiplex-19 inflammation ELISA. One-way ANOVA with Tukey's multiple comparisons test. *P<0.05, **P<0.01, #P<0.1.

4. AD-*B. dorei* alters Aβ plaque burden and microglia transcriptional profile

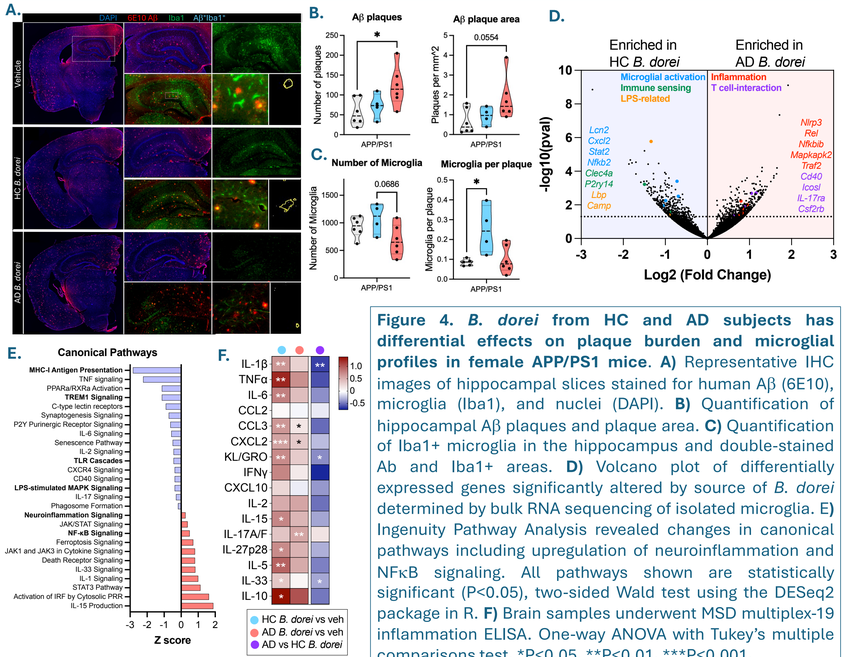


Figure 4. *B. dorei* from HC and AD subjects has differential effects on plaque burden and microglial profiles in female APP/PS1 mice. A) Representative IHC images of hippocampal slices stained for human Aβ (6E10), microglia (Iba1), and nuclei (DAPI). **B)** Quantification of hippocampal Aβ plaques and plaque area. **C)** Quantification of Iba1+ microglia in the hippocampus and double-stained Ab and Iba1+ areas. **D)** Volcano plot of differentially expressed genes significantly altered by source of *B. dorei* determined by bulk RNA sequencing of isolated microglia. **E)** Ingenuity Pathway Analysis revealed changes in canonical pathways including upregulation of neuroinflammation and NFκB signaling. All pathways shown are statistically significant (P<0.05), two-sided Wald test using the DESeq2 package in R. **F)** Brain samples underwent MSD multiplex-19 inflammation ELISA. One-way ANOVA with Tukey's multiple comparisons test. *P<0.05, **P<0.01, ***P<0.001.

5. Metabolomic and genomic profiling of *B. dorei* strains

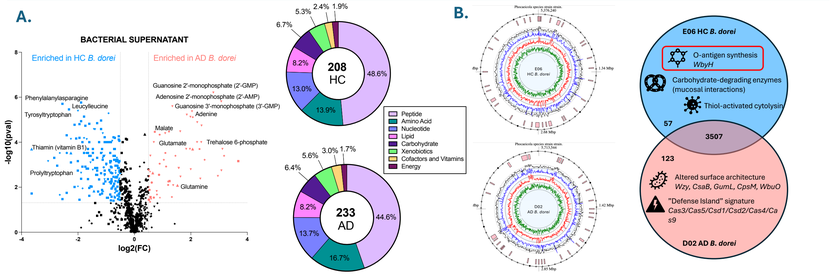


Figure 6. Metabolomics and genomic differences associated with disease status. A) Global metabolomics analysis on bacterial supernatant and the proportion of sub-pathways of produced metabolites per strain. **B)** Circular whole genome map of healthy control-E06 and AD-D02 *B. dorei*. AD-*B. dorei* harbors unique genes related to surface architecture and heightened defense. HC *B. dorei* contains the LPS-associated *WbyH* gene that is absent in the AD *B. dorei* strain.

6. Differential effect of LPS isolated from *B. dorei* strains

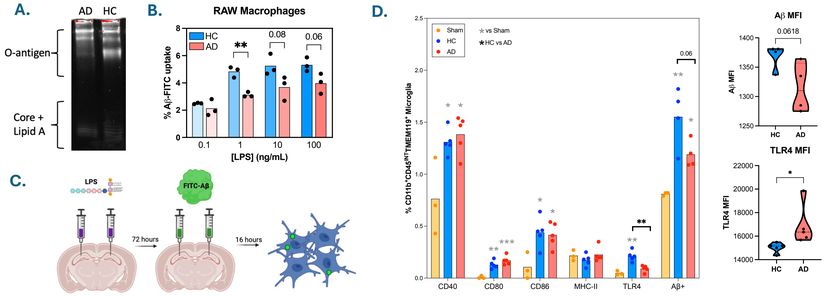
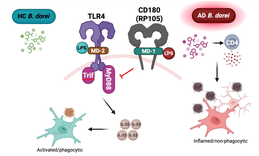


Figure 7. LPS isolated from an AD sample is structurally and functionally different from HC. A) SDS-page of isolated LPS from both strains reveal a structural alteration in the O-antigen chain. **B)** *In vitro* functional phagocytosis analysis. RAW macrophages primed with LPS for 24 hours before exposure to FITC-Aβ. Aβ uptake quantified via flow cytometry. **C)** *In vivo* functional phagocytosis analysis. Female, 8-week-old C57Bl/6 mice received bilateral intra-hippocampal injections of 2μg of LPS followed by a bilateral injection of FITC-Aβ. **D)** Microglia were harvested and underwent flow cytometry with surface staining for key activation markers and Aβ uptake. * P<0.05, ** P<0.01, *** P<0.001

Conclusions



Together, these findings demonstrate that strain-specific genomic and metabolic features of gut bacteria critically shape host immune responses and disease-relevant outcomes, underscoring the importance of strain-level resolution in microbiome research and highlighting the gut microbiota as a modifiable contributor to AD progression.